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(54) Title: GENETIC MARKERS FOR PIG LITTER SIZE

(57) Abstract

Disclosed herein are genetic markers for pig litter size, methods for identifying such markers, and methods of screening pigs to determine those more likely to produce larger litters. The markers are based upon the presence or absence of polymorphisms in the pig estrogen receptor gene. Preferably, the polymorphism is a restriction fragment length polymorphism (RFLP). A 4.3 kilobase fragment obtained by digesting pig genomic DNA with the restriction endonuclease Pvu II and detecting the fragments with a probe comprising a detectably labeled human estrogen receptor gene is associated with increased litter size.

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GENETIC MARKERS FOR PIG LITTER SIZE

FIELD OF THE INVENTION

This invention relates generally to the detection of genetic differences for reproductive efficiency among pigs and particularly to genetic markers useful for identifying pigs more likely to produce larger litter sizes.

BACKGROUND OF THE INVENTION

Reproductive efficiency, which can be defined as the number of pigs produced per breeding female, is the major limiting factor in the efficient production of pork. The number of pigs born alive in the United States averages approximately 9.5 pigs per litter. Heritability for litter size is low (10%-15%), and standard genetic methods of selecting breeding females on the basis of past litter size have not been effective. Therefore, there is a need for an approach that deals with selection for reproduction at the cellular or DNA level.

Chinese breeds are known for reaching puberty at an early age and for their large litter size. American breeds are known for their greater growth rates and leanness. Thus, it would be desirable to combine the best characteristics of both types of breeds, thereby improving the efficiency of U.S. pork production. These efforts would be greatly assisted by the discovery of genes or genetic markers that are associated with increased litter size in pigs.

Reproduction in mammals takes place in response to a chain of events that occur between the brain and the reproductive organs. The steroid hormones, such as estrogen, play a crucial role. Steroid hormones interact with cells and tissues, initiating a series of events that result in the ability to reproduce successfully.

In pigs, estrogen, which is produced mainly by the ovaries, has profound effects on the uterus, brain, and pituitary gland. Estrogens modulate the onset of puberty, reproductive behaviors, cyclic release of gonadotropins, and feeding behavior. The effects of estrogens take place as a result of the binding of estrogen to specific receptor proteins found in the nucleus of the estrogen-responsive cells. McEwen, et al., Recent Prog. Horm. Res., 38:41-92 (1982).

The gene responsible for coding for the human estrogen receptor has been identified, and it is publicly available from the American Type Culture Collection. See ATCC Catalog Sept. 1990, page 112, entry 57681. The probe name pOR3 and is 1.3 kb. Green et al., Nature (London) 320:134-139 (1986), incorporated herein by reference. The human gene is known to be polymorphic as a result of restriction fragment length polymorphism (RFLP) analysis. Castagnoli et al., Nucl. Acids Res., 15:886 (1987); Coleman et al., Nucl. Acids Res., 16:7208 (1988). The functional differences relating to these different genotypes are not well understood, but they have been implicated in increased spontaneous abortions in humans with breast cancer. Lehrer et al., The Lancet, 335:622-624 (March 17, 1990).

The estrogen receptor gene has been isolated and sequenced for other species, but not for pigs. Koike et al., Nucl. Acids Res., 15:2499-2513 (1987) reports the isolation and sequencing of a cDNA clone of the rat uterus estrogen receptor. The authors state that a comparison of rat, human, and chicken estrogen receptor sequences indicates the presence of three highly conserved regions, suggesting that these regions play important roles in estrogen receptor function.

In addition, Koike et al. Biochemistry 26:2563-2568 (1987) reports the partial characterization of the porcine estrogen receptor binding site. The paper reports a fragment of about 30 kDa that probably corresponds to the hydrophobic C-terminal-half region and has a greater than 90% homology with the corresponding rat, human, and chicken sequences.

RFLP analysis has been used by several groups to study pig DNA. Jung et al., Theor. Appl. Genet., 77:271-274 (1989) discloses the use of RFLP techniques to show genetic variability between two pig breeds. Polymorphism was demonstrated for swine leucocyte antigen (SLA) Class I genes in these breeds. Hoganson et al., Abstract for Annual Meeting of Midwestern Section of the American Society of Animal Science, March 26-28, 1990 reports on the polymorphism of swine major histocompatibility complex (MHC) genes for Chinese pigs, also demonstrated by RFLP analysis. Jung et al. Animal Genetics, 20:79-91 (1989) reports on RFLP analysis of SLA Class I genes in certain boars. The authors state that the results suggest that there may be an association between swine SLA/MHC Class I genes and production and performance traits. They further

state that the use of SLA Class I restriction fragments, as genetic markers, may have potential in the future for improving pig performance.

Prior to the present invention, RFLP analysis has not been applied to the pig estrogen receptor gene, which has not even been identified. The present invention overcomes these deficiencies. It provides genetic markers, based upon the discovery of polymorphism in the pig estrogen receptor gene, which relate to increased litter size in pigs. This will permit the screening and genetic typing of pigs for their estrogen receptor genes. It will also permit the identification of individual males and females that would be expected to produce a litter size larger than the average for their breed.

SUMMARY OF THE INVENTION

It is an object of the invention to provide a method of screening pigs to determine those more likely to produce larger litters.

Another object of the invention is to provide a method for identifying genetic markers for pig litter size.

A further object of the invention is to provide genetic markers for pig litter size.

Yet another object of the invention is to provide a kit for evaluating a sample of pig DNA.

Additional objects and advantages of the invention will be set forth in part in the description that follows,

and in part will be obvious from the description, or may be learned by the practice of the invention. The objects and advantages of the invention will be attained by means of the instrumentalities and combinations particularly pointed out in the appended claims.

To achieve the objects and in accordance with the purpose of the invention, as embodied and broadly described herein, the present invention provides a method for screening pigs to determine those more likely to produce a larger litter when bred. A sample of genomic DNA is obtained from a pig, and the presence or absence of a polymorphism in the estrogen receptor gene correlated with increased litter size is determined. Preferably, the polymorphism is a restriction fragment length polymorphism.

The presence or absence of a specific fragment or RFLP pattern is identified by the following steps. First, the genomic DNA is digested with a restriction endonuclease that cleaves the pig estrogen receptor gene in at least one place. Second, the fragments obtained from the digestion are separated, preferably by gel electrophoresis. Third, the fragments are detected with a probe capable of hybridizing to them. This generates a restriction pattern. Finally, the restriction pattern is compared to a known RFLP pattern for this gene that is correlated with increased litter size. The second pattern is one obtained by using the same restriction endonuclease and the same probe or an equivalent probe. Preferably, the probe is the human estrogen receptor gene.

In another embodiment, the invention comprises a method for identifying a genetic marker for pig litter

size. Male and female pigs of the same breed or breed cross or similar genetic lineage are bred, and the number of offspring produced by each female pig is determined. The polymorphism in the estrogen receptor gene of each pig is determined and associated with the number of offspring. Preferably, RFLP analysis is used to determine the polymorphism, and most preferably, the genomic DNA is digested with the restriction endonuclease Pvu II. For pigs of the Meishan breed, such analysis produces a 4.3 kilobase fragment associated with increased litter size.

The invention further comprises a kit for evaluating a sample of pig DNA. At a minimum, the kit is a container with one or more reagents that identify polymorphism in the pig estrogen receptor gene. Preferably, the reagent is a probe that hybridizes with the pig estrogen receptor gene or fragments thereof. Preferably, the probe is the human estrogen receptor gene. Preferably, the kit further contains a restriction enzyme that cleaves the pig estrogen receptor gene in at least one place.

The accompanying figure, which is incorporated in and constitutes a part of this specification, illustrates one embodiment of the invention and, together with the description, serves to explain the principles of the invention.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 shows RFLP analysis of Duroc (lane 1) and Chinese (lanes 2-16) pig DNA using the human estrogen receptor gene probe.

DETAILED DESCRIPTION OF THE INVENTION

Reference will now be made in detail to the presently preferred embodiments of the invention, which, together with the following examples, serve to explain the principles of the invention.

The invention relates to genetic markers for litter size in pigs. It provides a method of screening pigs to determine those more likely to produce a larger litter when bred by identifying the presence or absence of a polymorphism in the estrogen receptor gene that is correlated with increased litter size. As used herein, the term "increased litter size" means a significant increase in litter size above the mean of a given population.

The use of RFLPs is the preferred method of detecting the polymorphism. However, since the use of RFLP analysis depends ultimately on polymorphisms and DNA restriction sites along the nucleic acid molecule, other methods of detecting the polymorphism can also be used. Such methods include ones that analyze the polymorphic gene product and detect polymorphisms by detecting the resulting differences in the gene product.

RFLP analysis in general is a technique well-known to those skilled in the art. See, for example, U.S. Patents 4,582,788 issued April 15, 1986 to Erlich and 4,666,828 issued May 19, 1987 to Gusella, both of which are incorporated herein by reference. Broadly speaking, the technique involves obtaining the DNA to be studied, digesting the DNA with restriction endonucleases,

separating the resulting fragments, and detecting the fragments.

In the present invention, a sample of genomic DNA is obtained from a pig. Generally, peripheral blood cells are used as the source of the DNA. A sufficient amount of cells are obtained to provide a sufficient amount of DNA for analysis. This amount will be known or readily determinable by those skilled in the art. The DNA is isolated from the blood cells by techniques known to those skilled in the art.

In certain instances, it may be desirable to amplify the amount of DNA through the use of standard techniques, such as the polymerase chain reaction. This technique is described in U.S. Patents 4,683,195, issued July 28, 1987 to Mullis et al., 4,683,202, issued July 28, 1987 to Mullis, 4,800,159 issued January 24, 1989 to Mullis, et al., 4,889,818 issued December 26, 1989 to Gelfand, et al., and 4,902,624, issued February 20, 1990 to Columbus, et al., all of which are incorporated herein by reference.

The isolated DNA is then digested with a restriction endonuclease that cleaves or cuts DNA hydrolytically at a specific nucleotide sequence, called a restriction site. Such endonucleases, also called restriction enzymes, are well-known to those skilled in the art. For the present invention, one should be chosen that cleaves the pig estrogen receptor gene in at least one place, producing at least two fragments of the gene. A determination is made as to whether or not such fragments are polymorphic and if the polymorphism is associated with

litter size by techniques known in the art in conjunction with the teachings contained herein. Preferably, such restriction endonuclease is Pvu II. The amount of such enzyme to be added to the sample containing the pig DNA and the other appropriate conditions for treating the sample will be readily determinable to persons skilled in the art, given the teachings contained herein.

The restriction fragments are then analyzed by known techniques that generally involve either the separation of the fragments to obtain a particular pattern or the determination of different sizes of the fragments. The preferred technique for doing so is gel electrophoresis.

In this technique, the digested fragments are separated in a supporting medium by size under the influence of an applied electric field. Gel sheets or slabs, such as agarose or agarose-acrylamide, are typically used as the supporting medium. The sample, which contains the restriction fragments, is added to one end of the gel. One or more size markers are run on the same gel as controls to permit an estimation of the size of the restriction fragments. This procedure generally permits a degree of resolution that separates fragments that differ in size from one another by as little as 100 base pairs.

The separated fragments preferably are then denatured and transferred physically from the gel onto a filter, preferably a nylon membrane, by contacting the gel with the filter in the presence of appropriate reagents and under appropriate conditions that promote the transfer of the DNA. Such reagents and conditions are well-known to

those skilled in the art. Thus, the relative positions of the DNA fragments resulting from the separation procedure are maintained.

The next step involves the detection of the various categories of sizes of the fragments or, alternatively, the detection of a fragment of a particular size. The latter may be of particular interest because it is a genetic marker associated with increased litter size. In either case, the preferred technique is the use of a hybridization probe. Such a probe is an oligonucleotide or polynucleotide that is sufficiently complimentary or homologous to the fragments to hybridize with them, forming probe-fragment complexes. Preferably, the probe is a cDNA probe. The oligonucleotide or polynucleotide is labeled with a detectable entity. This permits the detection of the restriction fragments, to which the probes are hybridized. The probes are labeled by standard labeling techniques, such as with a radiolabel, enzyme label, fluorescent label, biotin-avidin label, and the like.

In the present invention, a cDNA for the human estrogen receptor gene is used as the polynucleotide of the probe. Preferably, the detectable moiety is ^{32}P or biotin-avidin. The inventors have discovered that this probe is sufficiently homologous to the pig estrogen receptor gene to bind to it and to the various fragments produced by restriction endonucleases. However, other substantially equivalent probes can be determined by those skilled in the art, given the teachings contained herein. As used herein, a probe that is "substantially equivalent" to the human estrogen receptor gene probe is one that hybridizes to the same polymorphic fragments of digests of the pig estrogen

receptor gene as does the human estrogen receptor gene probe when the same restriction enzyme is used. For example, particular fragments that are associated with pig litter size can be sequenced by known techniques, and synthetic probes can be prepared, also by known techniques.

In the preferred method, the probes are contacted with the nylon membrane that contains the restriction fragments for a sufficient period of time and under appropriate hybridizing conditions for the probes to hybridize to the fragments. The filter is then preferably washed to remove unbound probes and other unwanted materials.

The probe-fragment complexes, which are bound to the filter, are then detected by known techniques. For example, if the probe has been radioactively labeled (^{32}P), detection involves contacting the nylon membrane paper with a piece of radiosensitive film. Following an appropriate exposure period, the fragments of interest, including control fragments, are visualized.

The detection step provides a pattern, resulting from the separation of the fragments by size. Comparison of these fragments with control fragments of known size that have also been run on the same gel permits the estimation of the size of the various groups of fragments. The various polymorphisms in the pig estrogen receptor gene are then determined by comparison of the patterns produced by similar analysis of DNA from a number of different pigs. For some of the individual pigs, the patterns will differ from the usual pattern produced by most of the other pigs. This will be due to one or more restriction fragment length

polymorphisms, i.e., restriction fragments of a different length produced by the endonuclease that cuts the pig estrogen receptor gene. This indicates different base pair sequences in such pigs.

Once a particular RFLP has been identified, i.e., a restriction fragment of a particular length, a probe to this fragment may be constructed by the use of known techniques. This permits alternative and faster formats for detecting such polymorphism. For example, once the DNA is digested, a sandwich hybridization format can be used. Such an assay is disclosed in U.S. Patents 4,486,539 issued December 4, 1984 to Ranki, et al., and 4,536,419 issued January 7, 1986 to Ranki, et al., both of which are incorporated herein by reference. The sample is brought into contact with a capture probe that is immobilized on a solid carrier. The probe binds the fragment. The carrier is then washed, and a labeled detection probe is added. After additional washing, the detection probe is detected, thereby demonstrating the presence of the desired fragment.

Once the RFLP pattern has been determined or a particular polymorphic fragment has been determined, it is compared to a second, known RFLP pattern or fragment that is correlated with increased litter size. This second pattern or fragment has also been determined from the pig estrogen receptor gene, using the same restriction endonuclease as the first and the same probe or an equivalent thereof.

In an alternative embodiment of the invention, the restriction fragments can be detected by solution hybridization. In this technique, the fragments are first

hybridized with the probe and then separated. The separated probe-fragment complexes are then detected by detecting the detectable moiety in the probe as discussed above. Generally, such complexes are detected on the gel without transfer to filter paper.

Although the above methods are described in terms of the use of a single restriction enzyme and a single probe, the methods are not so limited. One or more additional restriction enzymes and/or probes can be used, if desired. Additional enzymes, breeds, and constructed probes can be determined through routine experimentation.

Genetic markers for pig litter size are determined as follows. Male and female pigs of the same breed or breed cross or derived from similar genetic lineages are mated. The number of offspring produced by each female pig is determined. RFLP analysis of the parental DNA is conducted as discussed above in order to determine polymorphisms in the estrogen receptor gene of each pig. The polymorphisms are associated with the number of offspring. At least 20 and preferably at least 40 female pigs are used in making these determinations. The number of times each female produces a litter (i.e., the parity) is at least 1 time. Preferably, the cycle of breeding and giving birth is repeated at least 2 times and most preferably 3 times. The preferred breeds of pigs are Meishan, Fengjing, Minzhu, Duroc, Hampshire, Landrace, Large White, Yorkshire, Spotted Poland China, Berkshire, Poland China, and Chester White. The most preferred breeds are Duroc, Hampshire, Landrace, Large White, Yorkshire, and Chester White. When this analysis is conducted for the Meishan breed and the polymorphism is determined by RFLP analysis using the

restriction endonuclease Pvu II, a 4.3 kilobase fragment is associated with increased litter size.

The reagents suitable for applying the methods of the invention may be packaged into convenient kits. The kits provide the necessary materials, packaged into suitable containers. Preferably, the containers are also supports useful in performing the assay. At a minimum, the kit contains a reagent that identifies a polymorphism in the pig estrogen receptor gene that is associated with an increased litter size. Preferably, the reagent is a probe that hybridizes with the pig estrogen receptor gene or fragments thereof. Preferably, both the probe and a restriction enzyme that cleaves the pig estrogen receptor gene in at least one place are included in the kit. In a particularly preferred embodiment of the invention, the probe comprises the human estrogen receptor gene, a pig estrogen receptor gene, or a gene fragment that has been labelled with a detectable entity and the restriction enzyme comprises Pvu II. Preferably, the kit further comprises additional means, such as reagents, for detecting or measuring the detectable entity or providing a control. Other reagents used for hybridization, prehybridization, DNA extraction, etc. may also be included, if desired.

The methods and materials of the invention may also be used more generally to evaluate pig DNA, genetically type individual pigs, and detect genetic differences in pigs. In particular, a sample of pig genomic DNA may be evaluated by reference to one or more controls. RFLP analysis is performed with respect to the pig estrogen receptor gene, and the results are compared with a control. The control is the results of a RFLP analysis of the pig

estrogen receptor gene of a different pig. Similarly, a pig may be genetically typed by obtaining a sample of its genomic DNA, conducting RFLP analysis of the estrogen receptor gene in the DNA, and comparing the results with a control. Again, the control is the results of RFLP analysis of the estrogen receptor gene of a different pig. Finally, genetic differences among pigs can be detected by obtaining samples of the genomic DNA from at least two pigs, identifying the presence or absence of polymorphism in the estrogen receptor gene, and comparing the results.

These assays are useful for identifying genetic markers relating to litter size, as discussed above, for identifying other polymorphisms in the estrogen receptor gene that may be correlated with other characteristics, and for the general scientific analysis of pig genotypes and phenotypes.

It is to be understood that the application of the teachings of the present invention to a specific problem or environment will be within the capabilities of one having ordinary skill in the art in light of the teachings contained herein. The examples of the products and processes of the present invention appear in the following examples.

EXAMPLE 1

Genetic Marker for Increased Litter Size in Meishan Pigs

Materials and Methods

The procedures for detecting the restriction fragment length polymorphisms (RFLPs) were as follows. Ten ml of sterile blood were obtained from each pig. Isolation of genomic DNA was then performed from white blood cells, followed by digestion by Pvu II restriction endonuclease, Southern blotting, and hybridization with the estrogen receptor gene probe as outlined in Flanagan et al., Immunogenetics 27:465-469 (1988), incorporated herein by reference. Molecular sizes of the restriction fragments were determined by comparison with molecular size markers for Hind III cut lambda DNA restriction fragments run in parallel on the hybridization gels. The estrogen receptor probe was a 1.3 kb probe from the estrogen receptor gene isolated from humans (locus ESR) that was obtained from The American Tissue Culture Collection NIH repository of Human and Mouse DNA Probes (ATCC No. 57681) and labeled with ^{32}P.

Results

Using the human estrogen receptor gene as a probe, we have used RFLP analysis on Chinese, American, and NIH miniature pigs to detect genetic differences for the homologous estrogen receptor locus in the pig. Our results reveal that there are at least four fragments that are polymorphic in the pig. These fragments are at 3.7, 4.3, 5.0, and 7.7 kb.

Further, we investigated whether the polymorphic restriction fragment patterns were related to litter size in our original 22 Meishan females. See Table 1. Based on our results, having the 4.3 kb fragment seems to increase the litter size, while not having the 4.3 kb seems to be a disadvantage. These data indicate that we have found a gene marker for litter size in Meishan pigs.

Table 1. Means and standard errors of litter size in Meishan females by parity and estrogen receptor fragment.

		Parity		
Fragments		1	2	3
With 4.3 kb	NB	12.7 ± .84	14.2 ± 1.16	16.3 ± .33
	NBA	12.4 ± .81	12.8 ± .92	15.0 ± 1.53
	N	7	5	3
Without 4.3 kb	NB	11.4 ± .71	11.4 ± 1.31	13.5 ± 1.84
	NBA	10.9 ± .65	10.2 ± 1.17	13.3 ± 1.79
	N	14	11	4

NB = Number Born, NBA = Number Born Alive, N = Number of litters

WE CLAIM:

1. A method of screening pigs to determine those more likely to produce larger litters comprising the steps of:

obtaining a sample of genomic DNA from a pig; and

determining the presence or absence of a polymorphism in the estrogen receptor gene associated with increased litter size.

2. The method of claim 1 wherein said polymorphism is a restriction fragment length polymorphism (RFLP).

3. The method of claim 2 wherein said step of identifying the presence or absence of said RFLP comprises the steps of:

digesting said genomic DNA with a restriction endonuclease that cleaves the pig estrogen receptor gene in at least one place;

separating the fragments obtained from said digestion;

detecting said fragments with a probe capable of hybridizing to said fragments, thereby generating a restriction pattern; and

comparing said pattern with a second RFLP pattern for the pig estrogen receptor gene obtained by using said

restriction endonuclease and said probe or equivalent thereof, wherein said second RFLP pattern is associated with increased litter size.

4. The method of claim 3 wherein said restriction endonuclease is Pvu II.

5. The method of claim 3 wherein said separation is by gel electrophoresis.

6. The method of claim 3 wherein said probe is the human estrogen receptor gene or a substantial equivalent thereof, said gene or substantial equivalent being labeled with a detectable entity.

7. The method of claim 3 wherein said step of comparing said restriction patterns comprises identifying specific fragments by size and comparing the sizes of said fragments.

8. The method of claim 3 further comprising the step of amplifying the amount of said pig estrogen receptor gene prior to said digestion step.

9. The method of claim 2 wherein said step of identifying the presence or absence of said RFLP comprises the steps of:

adding to said sample a restriction enzyme that cleaves the pig estrogen receptor gene into fragments; and

detecting the different sizes of said fragments.

10. The method of claim 9 wherein said step of detecting the different sizes of said fragments comprises the steps of:

separating said fragments by size using gel electrophoresis in the presence of a control DNA fragment of known size;

contacting said separated fragments with a probe that hybridizes with said fragments to form probe-fragment complexes; and

determining the size of the separated fragments by detecting the presence of the probe-fragment complexes and determining their relative positions with respect to said control DNA fragment.

11. The method of claim 2 wherein said step of identifying the presence or absence of said RFLP comprises the steps of:

adding to said sample a restriction enzyme that cleaves the pig estrogen receptor gene into fragments; and

determining the presence or absence of a DNA fragment of known size.

12. The method of claim 11 wherein said step of determining the presence or absence of said DNA fragment of known size comprises the steps of:

contacting said sample with a probe that hybridizes with said DNA fragment of known size to form probe-fragment complexes; and

detecting the presence or absence of said complexes.

13. A method for identifying a genetic marker for pig litter size comprising the steps of:

breeding male and female pigs of the same breed or breed cross or derived from similar genetic lineages;

determining the number of offspring produced by each female pig;

determining the polymorphism in the estrogen receptor gene of each pig; and

associating said number of offspring with said polymorphism.

14. The method of claim 13 wherein said polymorphism is determined by RFLP analysis.

15. The method of claim 13 wherein said breed is selected from the group consisting of Meishan, Fengjing, Minzhu, Duroc, Hampshire, Landrace, Large White, Yorkshire, and Chester White.

16. The method of claim 15 wherein said breed is Meishan and said polymorphism is determined by RFLP analysis.

17. The method of claim 16 wherein said RFLP analysis comprises digestion of genomic DNA with the restriction endonuclease Pvu II.

18. The method of claim 17 wherein said RFLP analysis produces a 4.3 kilobase fragment correlated with increased litter size.

19. A method for evaluating a sample of pig genomic DNA by reference to a control comprising the steps of:

conducting a RFLP analysis of the pig estrogen receptor gene; and

comparing the results of said analysis with said control, wherein said control comprises the results of a RFLP analysis of the pig estrogen receptor gene of a different pig from the pig that provided the sample of genomic DNA.

20. A method of genetically typing a pig comprising the steps of:

obtaining a sample of genomic DNA from said pig;

conducting RFLP analysis of the estrogen receptor gene in said DNA; and

comparing the results of said analysis with a control, wherein said control comprises the results of a

RFLP analysis of the estrogen receptor gene of a different pig.

21. A method of detecting genetic differences among pigs comprising the steps of:

obtaining samples of genomic DNA from at least two pigs;

identifying the presence or absence of a polymorphism in the estrogen receptor gene in said samples; and

comparing the results of said identification step.

22. The method of claim 21 wherein said step of identifying the presence or absence of a polymorphism comprises conducting RFLP analysis of said estrogen receptor gene.

23. The genetic marker identified by the method of claim 13.

24. A genetic marker for increased litter size in pigs comprising the 4.3 kilobase restriction fragment obtained by digesting DNA isolated from said pigs with the restriction endonuclease Pvu II.

25. A kit for evaluating a sample of pig DNA comprising, in a container, a reagent that identifies polymorphism in the pig estrogen receptor gene.

26. The kit of claim 25 wherein said reagent is a probe that hybridizes with the pig estrogen receptor gene or fragments thereof.

27. The kit of claim 26 wherein said probe comprises the human estrogen receptor gene or equivalent thereof labeled with a detectable moiety.

28. The kit of claim 25 further comprising a restriction enzyme that cleaves the pig estrogen receptor gene in at least one place.

29. The kit of claim 28 further comprising means for detecting said probe.

30. The kit of claim 29 further comprising control means.

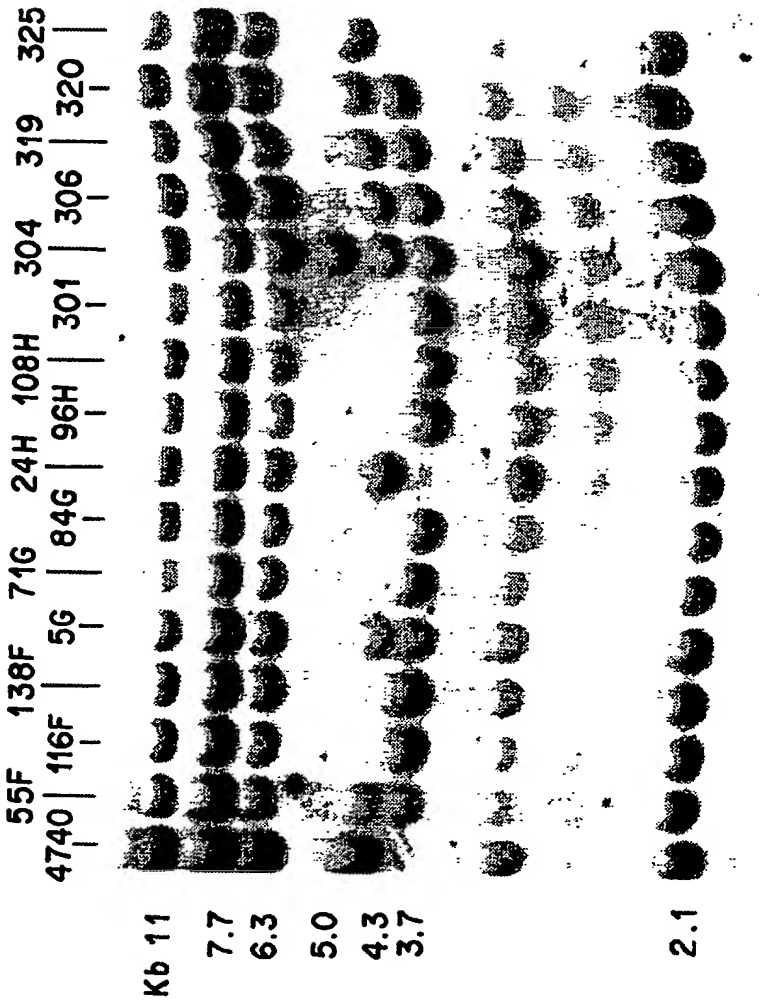
55F
138F
71G
24H
108H
304
319
325
4740
116F
5G
84G
96H
301
306
320
Kb 11
7.7
6.3
5.0
4.3
3.7
2.1

FIG. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/03160

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :IPC (5): C12Q 1/68; C12P 19/34; C07H 15/12
US CL :U.S. CL.: 435/6, 91; 536/27
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : U.S. CL.: 435/6, 91; 536/27

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, DIALOG,
search terms: RFLP's, Pig, Swine, estrogen receptor.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Nucleic Acids research, Vol. 16, No. 14, issued 1988, Coleman et al.,"Human estrogen receptor (ESR) gene locus: PasI dimorphism", page 7208, all document.	1-30
Y	Nucleic Acids Research, Vol. 15, No.2, issued 1987, Castagnoli et al.,"PvuII RFLP inside the human estrogen receptor gene" page 866, all document.	1-30
A	Biochemistry, Vol. 26, No.9, issued 1987, Koike et al.,"The steroid binding domain of porcine estrogen receptor", pages 2563-2568, all document.	1-30
A	Nucleic Acids Research, Vol. 15, No.6, issued 1987, Koike et al.,"Molecular cloning and characterization of rat estrogen receptor", pages 2499-2513, all document.	1-30
A	Animal Genetics, Vol. 20, issued 1989, Jung et al.,"Association of restriction fragment length polymorphisms of swine leucocyte antigen class I genes with production traits of Duroc and Hampshire boars", pages 79-91 all document.	1-30

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 20 JULY 1992	Date of mailing of the international search report 31 JUL 1992
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer MIGUEL ESCALLON PH.D. Telephone No. (703) 308-0376

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/03160

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Immunogenetics, Vol 21, issued 1985, Chardon et al.,"Restriction fragment length polymorphism of the major histocompatibility complex of the pig", pages 161-171, all document.	1-30
A	Immunogenetics, Vol. 27, issued 1988, Flannagan et al.,"RFLP analysis of SLA class I genotypes in Duroc swine", pages 465-469, all document.	1-30
A	Theoretical Applied Genetics, Vol. 77, issued 1989, Jung et al.,"Genetic variability between two breeds based on restriction fragment length polymorphisms (RFLPs) of major histocompatibility complex class I genes in the pig", pages 271-274, all document.	1-30